519

IN VITRO GENE KNOCKDOWN OF SOX9 AFFECTS CELL SURVIVAL AND OSTEOGENIC DIFFERENTIATION OF MESENCHYMAL STEM CELLS

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Purpose: The transcription factor Sox9 plays an important role in the regulation of skeletal growth and cartilage formation. It is absent in hypertrophic chondrocytes, where a misexpression in vivo causes lack of bone marrow, inhibition of cartilage resorption and failure of vascular invasion. Moreover, there is evidence that Sox9 is related to more biological processes then only to osteo-chondrogenesis. The aim of our study is to determine the role of Sox9 in undifferentiated rat mesenchymal stem cells (rMSC) for cell survival and its impact on osteogenic differentiation capacity.
Methods: rMSC were isolated from the femoral bone marrow of five week old CD-rats and cultured in monolayer for one passage. Cells were seeded at low density and transduced with a retroviral vector containing a Sox9-specific shRNA. After antibiotic selection, the Sox9 knockdown was determined with quantitative real-time PCR (qRT-PCR) and western blot. Downstream effects of the reduced Sox9 level in undifferentiated rMSC were investigated by qRT-PCR. Proliferation was quantified with a BrdU-ELISA based assay and the apoptotic activity with a caspase 3/7 assay. For transduced cells, subjected to osteogenic differentiation for up to 21 days, osteogenic marker gene expression was analyzed via qRT-PCR.
Results: A reproducible Sox9 knockdown >75% was generated on the mRNA and protein level. Downstream effects were a significant down regulation of Col1a1, Sox6, Sox5 Vegfa, Integrin alpha 11, Mmp13, Runx2 and Bcl-2 gene expression whereas Osteocalcin was strongly up regulated. The BrdU assay indicated ~ 20% reduction of proliferation and the caspase 3/7 assay displayed a ~ 12% increase in apoptotic activity after Sox9 silencing. Preliminary results from osteogenically differentiated rMSC showed an increased up regulation of Osteocalcin, Runx2, Vegfa and Mmp13 gene expression in cells with diminished Sox9 copies vs. control cells during and after termination of osteogenesis.
Conclusion: Based on these knockdown studies we conclude a positive effect of Sox9 on proliferation and on cell viability. We moreover postulate a possible interaction between Bcl-2 and Sox9 early in undifferentiated MSC prior to onset of chondrogenic/osteogenic differentiation, emphasizing an important role of Sox9 for viability and survival of multipotent precursor cells. Furthermore, our results may hint to an acceleration of osteogenic differentiation due to inhibition Sox9.

520

CHONDROGENIC POTENTIALITY OF HYPOXIA ON HUMAN BONE MARROW MESENCHYMAL STEM CELLS IN THREE DIMENSIONAL CULTURE

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Purpose: Human bone marrow mesenchymal stem cells (MSCs) are proved to be a good candidate cell source for treating cartilage defects. This is due to their ability to undergo chondrogenic differentiation after extensive expansion in vitro in three-dimensional (3-D) systems. Differentiation of MSCs into chondrocytes in vitro has been studied by means of exposure to exogenous growth factors, coculture with cartilage, and overexpression of specific genes to promote chondrogenic gene expression. In vivo, cartilage resides in a low oxygen microenvironnement and is exposed to hypoxic conditions (1-10% oxygen). Most in vitro tissue engineering strategies are performed within 20% oxygen environment, but it is becoming clear that changes in ambient oxygen levels exert a profound influence in chondrogenesis. Oxygen tension seems to be an important regulatory factor in chondrocytes culture that influences the expression of chondrogenic phenotypic genes and cartilage specific matrix formation. The aim of our study was to evaluate the influence of hypoxic conditions on chondrogenic differentiation of human mesenchymal stem cells in 3D culture (alginate beads supplemented with hyaluronic acid).
Methods: Mesenchymal stem cells issued from human bone marrow were expanded in monolayer under hypoxia (5% O2) or normoxia (21% O2) culture condition. After this monolayer expansion, mesenchymal stem cells were seeded in alginate bead (3D culture) enriched with hyaluronic acid and cultured in normoxia (21% O2) or in hypoxia (5% O2) with or without growth factor supplementation (TGFβ1, BMP-2). At D28, expression of cartilage interest genes and quality of synthesized extracellular matrix were analyzed respectively by using RT-PCRq, histology and immunohistochemistry (coll 1, coll 2).
Results: A strong increase of type 2 collagen expression was observed at D28 with growth factors. This increase was more important with BMP-2 combinated with TGF-β1 compared to TGF-β1 alone. BMP-2 alone was inefficient to increase collagen 2 basal expression observed with FBS or ITS. Interestingly, a significant increase of these inductions in hypoxia culture condition was observed. Hypoxia 3D culture condition enhanced significantly sox 9 and aggrecan expressions. COMP and versican expressions were not significantly influenced by hypoxia. Osteocalcin and alkaline phosphatase, exerting a low basal gene expressions, were not modified by hypoxia. Moreover, monolayer culture (expansion phase) under hypoxia increased the induction of Coll 2 chondrocytic gene, particularly type 2B. Histologically, hypoxia induces an abundant newly synthetized extracellular matrix, rich in proteoglycan and collagen, mainly type 2.
Conclusions: Chondrogenic differentiation of mesenchymal stem cells in combination with growth factors in alginate beads is significantly favored by their exposition to hypoxia condition in monolayer and in 3D culture conditions. Moreover, the preconditioning of monolayer culture under hypoxia potentializes the induction of type 2 collagen in 3D culture.

521

SEARCHING FOR A WELL-DEFINED AND EFFICIENT METHOD FOR IN VITRO DIRECTING STEM CELL DIFFERENTIATION INTO THE CHONDROGENIC LINEAGE

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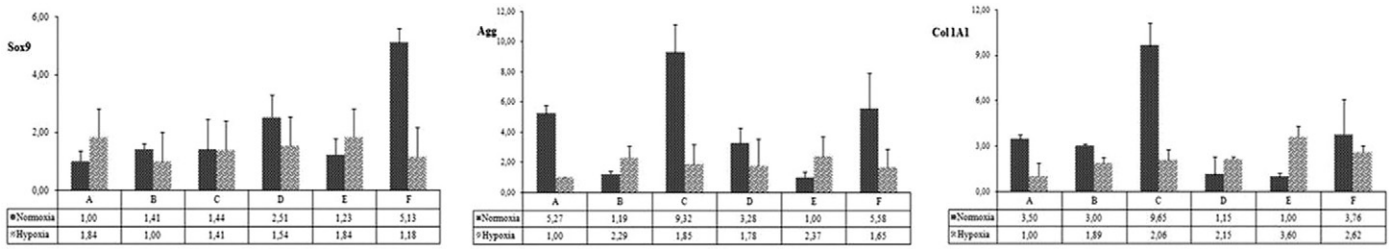
Purpose: The use of autologous or allogenic stem cells is suggested as an alternative therapeutic approach for treatment of cartilage defects. The aim of this study was to develop a well-defined and efficient method for *in vitro* directing bone marrow mesenchymal stem cells (BM-MSCs) differentiation into the chondrogenic lineage both in normoxic and in hypoxic conditions.
Methods: At the third passage, BM-MSCs (n= 3) were characterized by flow cytometry and differentiated for 14th days towards chondrogenic lineage by the use of three different growth factors: A (Control: DMEM+15% KO serum), B (TGFβ-3 1ng/ml), C (TGFβ-3 10ng/ml), D (BMP-2 10ng/ml), E (BMP-7 10ng/ml) and F (TGFβ-3 1ng/ml+ BMP-2 10ng/ml+ BMP-7 10ng/ml). Chondrogenic differentiation of BM-MSCs was confirmed by the use of histochemical, immunohistochemical and qPCR techniques.
Results: BM-MSCs displayed similar surface antigen expressions. As shown in the graphic, gene expression levels of Sox9, Agg and Col1A1 was measured in the different chondrogenic culture mediums (Fig. 1). The gene expression of Col2A1 was detectable only in F and C medium.



Abstract 521 – Figure 1. For each gene in each culture condition, the reference value 1 is given to the lower expression level.